

## INSIGHTS

### Refusing to TAP out: 16 new human TEIPPs identified

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In this issue of *JEM*, Marijt et al. (<https://doi.org/10.1084/jem.20180577>) report their discovery of 16 novel human TAP-independent TEIPP peptides, whereas only one had been previously identified. This opens the door to new therapeutic options for patients with TAP-deficient tumors.

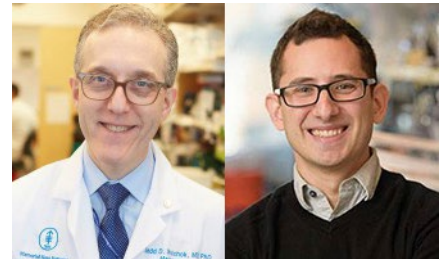
Cancer therapies must contend with drug resistance, and immunotherapy is no exception. In this case, resistance is often the result of defects in the antigen presentation machinery (APM) required for the immune system to distinguish between a cancer cell and any other healthy cell in the body. One of the main components of the APM is the transporter associated with antigen processing, also known as TAP (Neeffjes et al., 2011). Work done by Marijt et al., published in this issue, characterizes 16 novel antigens in humans that are presented independently of TAP, which could be potential targets for immunotherapy in patients with TAP-deficient tumors.

CD8<sup>+</sup> T cells, or CTLs, target tumor cells by recognizing small 8–11-aa-long peptides complexed with MHC-I molecules at the surface of the tumor cells in a phenomenon called antigen presentation. In order for this presentation to occur, proteasomes in the cytoplasm degrade full-length proteins into smaller peptides, which are subsequently transported into the ER, where they are loaded onto nascent MHC-I molecules. Once stably assembled, this peptide:MHC-I complex egresses from the ER to the cell surface, where it can be recognized by peptide-specific CTLs. For the vast majority of peptides, this transport from the cytoplasm into the ER and loading onto MHC-I is performed by TAP (Suh et al., 1994) and is a crucial and rate-limiting step for successful antigen presentation. Unsurprisingly, some tumor cells under pressure from the immune system, whether through checkpoint blockade therapy or a spontaneously occurring immune response, acquire defects in TAP that allow them to evade the immune system and dominate the remaining tumor (Sharma et al., 2017). Ultimately, patients who develop TAP-deficient

tumors are no longer viable candidates for T cell-based immunotherapy.

However, a class of peptides known as TEIPP (T cell epitopes associated with impaired peptide processing) can enter the ER independently of TAP (van Hall et al., 2006) by virtue of specific features in the protein sequence that lead to alternative processing (Oliveira and van Hall, 2015). TEIPP peptides are derived from non-mutated housekeeping proteins found in multiple cell types. Importantly, for reasons still subject to further investigation, TEIPP peptides are uniquely presented by TAP-deficient cells, but not by TAP wild-type cells (Marijt et al., 2018b). As a result, TEIPP-specific T cells are not deleted in the TAP-normal thymus (Doorduijn et al., 2016). Therefore, TEIPPs can reasonably be considered as a separate class of neoantigens, and targeting TEIPP through vaccines and TCR-modified CTLs is an attractive approach to immunotherapy for patients whose tumors develop defects in TAP. In previous preclinical studies in mouse models, also by the van Hall group, TCR-modified CTLs targeting TEIPPs and TEIPP-specific vaccines were shown to be effective in controlling tumor growth in TAP-deficient, but not TAP-normal, tumor cells (Chambers et al., 2007; Doorduijn et al., 2016). These proof-of-principle experiments demonstrated that targeting TEIPPs could, in fact, be used as a potential immunotherapy against TAP-deficient tumors, at least in mice. However, far less is known about TEIPPs in humans, as only one has been characterized at the molecular level (El Hage et al., 2008). Consequently, to move forward with testing TEIPPs as viable targets in humans, it was necessary to identify more TEIPPs.

This is what Marijt et al. (2018a) accomplished by developing a systematic hybrid



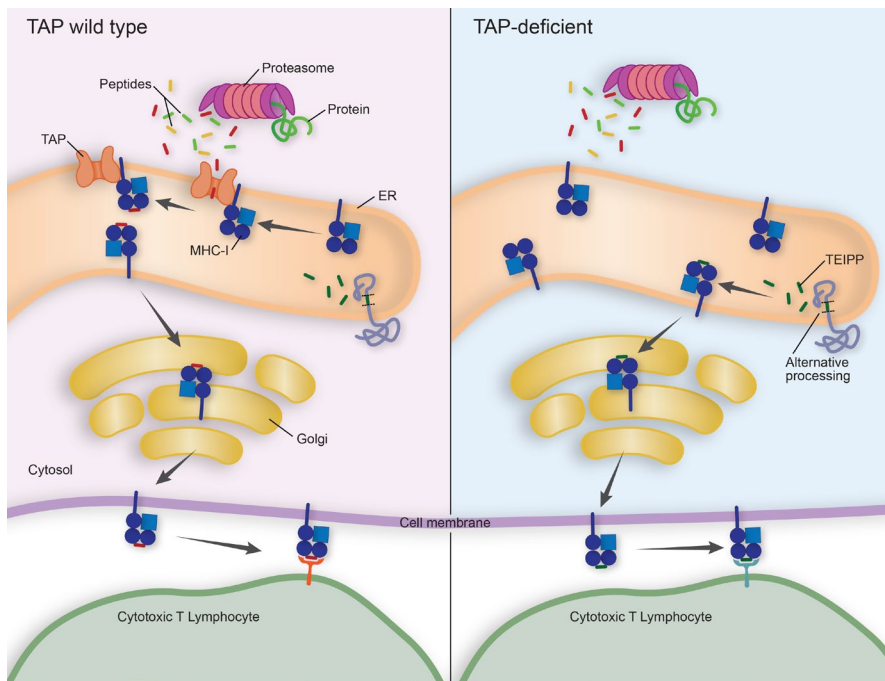
Insights from Jedd Wolchok and Mathieu Gigoux.

forward-reversed immunology screen, whose results are presented in this issue. Their screen involved examining the entire human proteome in silico to generate a list of candidate peptides based on two known mechanisms of alternative processing. The list was further refined for peptides predicted to bind to the most common HLA class I molecule in the Caucasian population, HLA-A\*02:01 (HLA-A2). They then compared this list to presented peptides eluted from tumor samples, but not healthy tissue, to get a shortlist of 40 candidate HLA-A2 TEIPP peptides. These peptides were then tested to determine which could promote the in vitro expansion of HLA-A2 CD8<sup>+</sup> T cells from healthy donors, a proxy for immunogenicity in the body. In total, they found that of the 40 candidate peptides, 16 could promote detectable CD8<sup>+</sup> T cell expansion in at least one of three tested samples, and of these, 14 peptides did so in at least three of seven samples tested. Next, they focused their attention on one TEIPP peptide that responded in all 12 samples tested, a peptide that is encoded by the LRPAP1 protein. Importantly, they found that LRPAP1-specific CD8<sup>+</sup> T cell clones produced more cytokines when cultured with TAP-deficient lymphomas, melanomas, and renal and colon carcinomas expressing LRPAP1, but

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T cell epitopes associated with impaired peptide processing, or TEIPPs, are peptides derived from non-mutated self-peptides, which behave as neoantigens in TAP-deficient tumor cells. In TAP wild-type cells, proteins are degraded by proteasomes in the cytosol into smaller peptides, which are transported and loaded onto MHC-I by TAP. The peptide:MHC-I complex subsequently translocates to the cell surface, where it can be recognized by CTLs. TEIPPs, which enter the ER through TAP-independent alternative processing, do not bind to MHC-I in TAP wild-type cells for reasons still under investigation. However, in TAP-deficient tumor cells, TEIPPs successfully complex with MHC-I and can be recognized by TEIPP-specific CTLs, which are not eliminated by the thymus.

not with normal tissue of the same origins expressing equal levels of LRPAP1. Likewise, when these clones were cultured with an LRPAP1-expressing melanoma cell line, artificially knocking out TAP in the tumor cells elicited a stronger response than their unsilenced counterpart. Together, not only did this study identify 16 novel TEIPPs in humans, but it shows encouraging *in vitro* data suggesting that TEIPP-specific immune responses can indeed target TAP-deficient tumor cells in humans, as well as in mice.

In light of an expanded list of human TEIPPs and positive confirmatory *in vitro* results demonstrating that human TEIPPs appear to behave in a similar manner to their mouse counterparts, the question remains: Are we ready to begin testing in humans? TEIPPs certainly appear to offer a scientifically rational option to patients with TAP-deficient tumors. In addition, their non-mutated nature suggests that targeting TEIPPs could be an “off the shelf” therapy and would not be as resource intensive as other strategies, such as those targeting neoantigens. However, other factors must be taken into consideration. First, while they found that 16 peptides could indeed elicit CD8<sup>+</sup> T cell expansion *in vitro*, in only one of these cases were the resulting

CD8<sup>+</sup> T cell clones confirmed to also react to TAP-deficient cells expressing endogenous levels of the target protein, and even this interaction was measured in a reductionist *in vitro* culture. Second, as self-peptides are being targeted, autoimmunity will also inevitably be a worry before and during the initial testing phase. Thankfully, preclinical studies in mice have yet to show any signs of autoimmunity (van Hall et al., 2006; Chambers et al., 2007; Doorduyn et al., 2016, 2017), and the current study also shows that human TEIPP-specific CD8<sup>+</sup> T cell clones against LRPAP1 did not cross-react with healthy cells expressing LRPAP1 in a coculture system. However, as patients in the clinic are usually more heterogeneous than mice or cell lines, autoimmunity will ultimately be hard to assess until the therapy is attempted in earnest. Third, patient selection will have to be properly considered. The past decades are replete with unsuccessful clinical trials that, with the benefit of hindsight, likely failed due to poor patient selection, especially those requiring very specific conditions to work. In the case of melanoma, only 1–2% of patients have deleterious mutations in TAP1 or TAP2, yet epigenetic silencing results in low TAP1 expression in metastatic melanoma in a

high frequency of cases (Garrido et al., 2016; Ritter et al., 2017). Should these patients also be included in such a trial? How low do TAP levels need to be before TEIPPs begin to be presented and therefore become a viable target? Low levels of TAP could also be the result of low levels of inflammation, which would also correlate with low levels of other members of the APM, including MHC class I molecules. As an immune response against TEIPPs would likely require sufficient levels of other APM proteins, these tumors should likely not be categorized as TAP-deficient tumors. In other words, one can imagine that being too generous with this criterion would inevitably lead to many nonresponding patients. Therefore, it will be important to catalog in detail the nature of the TAP deficiency before any TEIPP therapy. Nevertheless, when all data from mice and now human studies are taken together, there are more reasons to begin testing in humans than not.

Of course, there still many unknowns that will be encountered in the clinic that haven't been addressed by current experiments, such as highly immunosuppressive tumor microenvironments and clonally heterogeneous tumors, or alternatively, whether other immunotherapies can be synergistic or even detrimental to the therapy, to name a few. And “unknown unknowns” always lurk around the corner. However, while there is much more work left to be done, this work by Marijt et al. (2018a) is a starting pistol for the race to bring a viable new immunotherapeutic paradigm to patients with tumors bearing TAP defects.

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